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ENHANCEMENT OF ANTIOXIDANT ABILITIES AND THE LIPOXYGENASE AND XANTHINE OXIDASE INHIBITORY ACTIVITY OF BROCCOLI SPROUTS BY BIOTIC ELICITORS

Urszula Gawlik-Dziki, Michał Świeca, Danuta Sugier
University of Life Sciences in Lublin

Abstract. *Saccharomyces cerevisiae* (SC) and *Salix daphnoides* bark (SD) water extracts were used for improving health-beneficial capacity of broccoli sprouts. The SC dilutions tested were 0.1, 0.5 and 1% (w/v), the SD concentration was 1% (w/v). Elicitation significantly modified the phenolics and flavonoid content, antiradical and chelating abilities, lipoxygenase (LOXI) and xanthine oxidase inhibitory activity (XOI). SC proved to be a better elicitor at a low concentration of 0.1% inducing antiradical, LOXI, XOI activities of both – hydrophilic and lipophilic extracts. SD water extract effectively induced lipophilic compounds with XOI activity and hydrophilic compounds with LOXI and antiradical activity. In most cases better results were obtained after one step induction. In the light of proved data nutaceutical value of non-processed food (broccoli sprouts) could be effectively enhanced by elicitation.

Key words: broccoli, elicitors, phenolics compounds, antioxidants, inhibition

ABBREVIATIONS USED

SC – *Saccharomyces cerevisiae*, SD – *Salix daphnoides* bark, LOXI – lipoxygenase inhibitory activity; XOI – xanthine oxidase inhibitory activity; ROS – reactive oxygen species; PBS – phosphate saline buffer

INTRODUCTION

Reactive oxygen species (ROS) can react with various cellular components such as polyunsaturated fatty acids, carbohydrates, proteins, and nucleic acids, leading to oxidative degradation and subsequent senescence, diseases, and accelerated ageing [Hodges

Corresponding author – Adres do korespondencji: Urszula Gawlik-Dziki, Department of Biochemistry and Food Chemistry, University of Life Sciences in Lublin, Skromna Str. 8, 20-704 Lublin, Poland, tel. (+ 48) 81 462 33 27, fax: (+48) 81 462 33 24, e-mail: urszula.gawlik @up.lublin.pl

and Lester 2006]. Creating ROS is bound, inter alia, with lipoxygenase (LOX) and xanthine oxidase (XO) activity. LOX catalyzes oxygenation of polyunsaturated fatty acids containing a cis, cis-1,4- pentadiene system to hydroperoxides. Three LOXs (5-LOX, 12-LOX, and 15-LOX) have been reported to be present in human tissues. LOXs and their products have also been reported to be important regulators in the proliferation and apoptosis of cancer cell lines. Inhibitors of arachidonic acid metabolism also inhibited tumor promotion in animal models [Juntachote and Berghofer 2005, Hong et al. 2001]. XO catalyses the oxidation of hypoxanthine and xanthine to uric acid. During the reoxidation of xanthine oxidase, molecular oxygen acts as an electron acceptor, producing superoxide radical and hydrogen peroxide. XO is considered to be an important biological source of superoxide radicals. These and other reactive oxygen species are involved in many pathological processes such as inflammation, atherosclerosis, cancer, etc. [Ferraz Filha et al. 2006].

It has been widely reported that sprouts provide higher nutritive value than raw seeds and their production is simple and inexpensive. Sprouts have long been used in the diet as health food and recent shows that in addition to being a good source of nutritional compounds, they also have important phytochemicals with disease preventive and health promoting properties [Randhir et al. 2004]. There is epidemiological evidence that diets rich in *Brassica* vegetables (broccoli, cabbage, cauliflower, radish etc.) are associated with a lower risk of lung and colorectal cancer. Among vegetables, broccoli has been recognized as an important source of various biologically active compounds that may work synergistically to aid prevention of cancer [Moreno and 2006, Martinez-Villaluenga et al. 2010].

Seed and seedlings treatments for improving plant vigour are being developed and used. Increasingly, commercial seed treatment approaches are beginning to view seed treatments as a means to increase substantially the value of the seed and to improve plant growth and productivity [Andarwulan and Shetty 1999]. Improvement of nutritional and nutraceutical value of plant/sprouts will be beneficial for human health. The aim can be reached in two main ways: genetic manipulation and modification of metabolism by elicitors. Genetic engineering of plant food has proven to be controversial. Modification of chemical composition and selected bioactivities of plant food by elicitors is cheapest and socially acceptable. Sprouting seeds may be regarded as a natural bioreactor or biotechnological module.

The hypothesis of this research is that the nutraceutical value of broccoli sprouts can be increased by treatment of seedlings with natural elicitors. The biotic elicitors: autoclaved cultures of yeast (*Sacchcaromyces cerevisiae*) and water extracts of *Salix daphnoides* bark were used. The parameters measured to characterize the effect of that elicitor were: total phenolics and flavonoids content, abilities to lipoxygenase (LOX) and xanthine oxidase (XO) inhibition, antiradical activity, chelating power.

MATERIALS AND METHODS

Dry seeds of broccoli (var. Wiarus) were purchased in local garden shop. Seeds were sterilized with 1% sodium hypochloride for 5 min. Sodium hypochloride was removed

by washing with sterile water. After disinfection, seeds were allowed to imbibe water at 25°C for 6 h. Seeds were dark germinated in sterile Petri plates with humidified Whatman Grade No. 2 Filter Paper at 25°C. The plates were covered with aluminum foil and the seeds were germinated for 6 days. The germinating seeds were watered with distilled water, 6 ml/24 h. For improving biological activities sprouts were treated with elicitors. One part of three-days-old seedlings (Ix1) was elicited only once, the second part (Ix3) was induced three 3 times (in 2^{nd}, 3^{rd}, at 4^{th} day of germination). Elicitors used in this study were prepared as follow: 1) Yeast *Saccharomyces cerevisiae* L. (SC). Yeast was purchased in local supermarket, dissolved in distilled water and autoclaved. Elicitors were tested in three different concentrations 0.1, 0.5 and 1% (w/v). 2) *Salix daphnoides* (SD). Bark of *Salix daphnoides* (SD) (obtained from ecological farm, Poland) were dried, pulverized in laboratory meal and extracted with boiling water. The final concentration of elicitor was 1 g/100 ml (w/v).

6-day-old sprouts were freeze dried and stored at -20^{o}C until further analyses. For determination of chemical composition and antioxidant activity of sprouts two kinds of extracts were prepared: 1) Chemical extracts: One gram of sprouts was homogenized with 7 ml of methanol: water (1:1, v/v) solution . Samples were shaken at room temperature for 60 min. After that, extracts were centrifuged at 13 000 for 10 min. Extraction was repeated, supernatants were collected and used for further analyses of lipophilic compounds. 2) Buffer extracts: One gram of sprouts was homogenized wit 7 ml of PBS buffer (phosphate buffered saline pH = 7.2) and shaken at room temperature for 60 min. After that extracts were centrifuged at 13 000 for 10 min. Extraction was repeated, supernatants were collected and used for further analyses of hydrophilic compounds.

The amount of total phenolics was determined using Folin-Ciocalteau reagent [Singleton and Rossi 1965]. Total flavonoids content was determined according to the method described by Bahorun et al. [2004]. Lipoxygenase inhibitors activity was determined spectrophotometrically according to the method described by Axelroad et al. [1981]. The xanthine oxidase inhibitors activity was measured spectrophotometrically, based on the procedure reported by Sweeney et al. [2001]. To determine the mode of inhibition by studied extracts, Lineveawer-Burk plot analysis was performed. The free radical scavenging activity of lipophilic and hydrophilic compounds was measured according to Brand-Williams et al. [1995] and Re et al. [1999], respectively. Chelating power was determined by the method of Guo et al. [2001].

All experimental results were mean ± S.D. of three parallel experiments (n = 9). The obtained data was subjected to a statistical analysis and the consequent evaluations were analyzed for a variance analysis. The statistical differences were estimated through Tukey's test. Statistical tests were evaluated by using the Statistica 6.0 software (StatSoft, Inc., Tulsa, USA). All the statistical tests were carried out at a significance level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

Several studies have recently demonstrated that *Brassica* vegetables, such as broccoli (*Brassica oleracea* var. *italica*) and radish (*Raphanus sativus*) may provide protection against oxidative damage and, consequently, prevent chronic diseases, mainly due high active compounds eg. polyphenols concentration [Martinez-Villaluenga et al. 2010]. Phenols have been widely studied and confirmed to possess diverse biological activities, which could be beneficial to human health. Broccoli sprouts were a good source of both, hydrophilic and lipophilic, phenolic compounds. Taking into account different extraction systems should be noted that PBS extraction was more effective than 50% methanol (v:v), (tab. 1). It could be explained by the fact that Folin-Ciocalteau reaction is not specific, other hydrophilic compounds such as ascorbic acid, peptides. It could also be due to higher water solubility of phenolic glycosides widely distributed in broccoli sprouts. It is worth noting that stimulation by SC and SD extracts

Table 1. Total phenolics and flavonoids content in the elicited broccoli sprouts
Tabela 1. Zawartość związków fenolowych i flawonoidów ogółem w indukowanych elicitorami kiełkach brokułu

Sample Próba	Total phenolics Związki fenolowe ogółem CAE mg/g f.m.				Total flavonoids Flawonoidy ogółem QE mg/g f.m.			
	hydrophilic hydrofilne		lipophilic lipofilne		hydrophilic hydrofilne		lipophilic lipofilne	
	Ix1	Ix3	Ix1	Ix3	Ix1	Ix3	Ix1	Ix3
Control Kontrola	14.05±0.05 Ba	14.05±0.05 Bc	8.86±0.03 Ab	8.86±0.03 Ab	2.55±0.02 Ba	2.55±0.02 Bab	0.80±0.01 Aa	0.80±0.01 Aa
SC 0.1%	14.15±0.25 Aab	15.98±0.98 Ca	14.63±0.45 Aa	12.98±0.35 Ba	2.04±0.05 Cc	2.63±0.15 Da	0.71±0.03 Ab	1.29±0.10 Bb
SC 0.5%	14.12±0.31 Cab	15.50±0.56 Da	10.37±0.37 Ac	12.44±0.38 Ba	2.45±0.09 Aa	2.46±0.10 Ab	0.61±0.02 Bc	0.58±0.03 Bc
SC 1%	15.67±0.59 CBc	15.54±0.23 Ca	14.53±0.56 ABa	14.74±0.45 Ac	2.30±0.12 Cd	2.51±0.10 Cab	1.19±0.09 Bd	0.80±0.04 Aa
SD 1%	15.06±0.89 Bcb	12.58±0.89 Ab	18.14±0.21 Cd	18.10±1.02 Cd	1.79±0.05 Cb	2.85±0.13 Dc	0.72±0.08 Aab	1.19±0.06 Bb

Different capital letters in the line for the same elicitors or control indicate statistical differences ($p < 0.05$)
Wartości średnie oznaczone w wierszach dużymi literami różnią się statystycznie istotnie przy $p < 0,05$
Different small letters in the columns for the same treatment indicate statistical differences ($p < 0.05$)
Wartości średnie oznaczone w kolumnach małymi literami różnią się statystycznie istotnie przy $p < 0,05$

significantly increased level of lipophilic phenolic compounds (tab. 1). The most effective elicitor was SD water extract in concentration of 1%. In majority of cases treatment of sprouts with biotic elicitors did not change significantly the level of hydrophilic phenols (tab. 1). Maximal increase of total phenolics was about 13%. It is interesting that three step SD stimulation caused significant decrease of total phenolics content (tab. 1).

It could be seen that one step treatment by SD extract caused significant decrease of PBS extractable flavonoids level. For lipophilic extracts significant increase of flavonoids content was observed after three step treatments by SC 0.1% and SD 1% extracts. On the other hand single and three step induction by SC 0.5% caused decrease of their level (tab. 1).

Natural elicitors are able to increase of phenolic content in plant sprouts. Andarwulan and Shetty [1999] tested acetyl salicylic acid (ASA) and fish protein hydrolyte (FPH) as natural elicitors for improving of pea (*Pisum sativum*) seed vigour response. When seeds were treated with ASA during pre-hydration the total phenolic content of pea seedlings increased slightly. These results were confirmed in this study. In our studies SD (natural source of ACA) treatment increased significantly lipophilic phenolic content in both experiments, whereas in hydrophilic compound case, significant decrease was observed after three step induction (tab. 1). Influence of peptide and phytochemical elicitors on phenolic stimulation in dark germinated mung bean sprouts was studied by Randhir et al. [2004] . They stated that 10ml/l fish protein hydrolisate (FPH) elicited phenolic production by 46% (in comparison to control). Also, lactoferrin (LF) at concentration 250 ppm caused an increase of phenolics level (elevation about 35% in comparison to control). Stimulation of sprouts by oregano extract (OE) caused reduction of phenolic content on days 4 and 5. It should be noted that during the later stages of germination on days 7 and 8, the treatments in the range of 2 ml to 5 ml/l OE caused an increase of phenolic content (approximately 70–80% higher compared with control).

As being present elicitors used in these studies also effected on phenolic content of sprout. Yeast extract was a source of polysaccharides, whereas SD extract was a source of salicylic acid derivatives. Experimental systems used by citied investigators based on stimulation of sprouts metabolism on the seeds level. The sprouts in our studies were treated in differ manner. By the fact that biochemical pathways in growing seedling are already working we supposed that stimulation sprouts may be most effective that treatment of no germinated seeds. Additionally, there are some reports about negative action of elicitors on seed's enzymes – they can act as inhibitors of keys pathways involved in mobilization of reserved materials during growth of seedlings.

Human and rabbit reticulocyte 15-lipoxygenase (15-lipoxygenase-1) and the leukocytetype 12-lipoxygenases (12/15-lipoxygenases) of pig, beef, mouse and rat constitute a particular subfamily of mammalian lipoxygenases (reticulocyte type lipoxygenases) with unique properties and functions. They catalyze enzymatic lipid peroxidation in complex biological structures *via* direct dioxygenation of phospholipids and cholesterol esters of biomembranes and plasma lipoproteins. Moreover, they are a source of free radicals initiating non enzymatic lipid peroxidation and other oxidative processes [Schewe 2002]. LOXs and their products have also been reported to be important regulators in the proliferation and apoptosis of cancer cell lines. It was observed that the expression of 15-LOX was increased significantly in human epithelial cells. These results suggest that the regulation of arachidonic acid metabolism is important in the prevention of many types of cancer, especially cancers of the digestive tracts [Hong et al. 2001].

Therefore an attempt was made to study the lipoxygenase inhibitory activities of commonly consumed vegetables. Since extracts of the vegetables tested show high

activity both as radical scavengers and lipoxygenase inhibitors it is possible that these activities may be involved in their putative medicinal properties. The interaction of flavonoids with mammalian 15-lipoxygenase-1 merits particular attention, since this enzyme is a potential target for the health-preserving effect of flavonoids [Sadik et al. 2003]. Inhibition of soybean 15-LOX is generally regarded as a predictive for inhibition of the mammalian enzyme [Maiga et al. 2006].

Table 2. Effect of elicitors on sprouts ability to inhibit enzyme activity, expressed as IC50 (mg/ml), n = 9

Tabela 2. Wpływ elicitorów na zdolność kiełków do inhibicji aktywności enzymów, IC_{50} (mg/ml), n = 9

Sample Próba	LOX inhibitory activity Inhibicja LOX				XO inhibitory activity Inhibicja XO			
	hydrophilic hydrofilne		lipophilic lipofilne		hydrophilic hydrofilne		lipophilic lipofilne	
	Ix1	Ix3	Ix1	Ix3	Ix1	Ix3	Ix1	Ix3
Control Kontrola	0.88±0.02 Ab	0.88±0.02 Ac	0.92±0.04 Ac	0.92±0.04 Aa	1.86±0.06 Aa	1.86±0.06 Aa	3.64±0.10 Bd	3.64±0.10 Bd
SC 0.1%	0.71±0.02 Ba	0.78±0.03 Aa	0.84±0.05 Ad	0.82±0.03 Ad	1.80±0.07 Aa	1.80±0.08 Aa	2.38±0.21 Ba	2.96±0.21 Cc
SC 0.5%	0.82±0.04 ABbc	0.79±0.02 Aa	0.85±0.06 Cac	0.96±0.05 Cac	1.81±0.09 Aa	1.84±0.10 Aa	3.19±0.09 Cc	2.48±0.18 Ba
SC 1%	0.77±0.05 Aac	0.72±0.01 Ab	1.19±0.08 Bbc	1.09±0.08 Bbc	1.79±0.12 Aa	1.87±0.12 Aa	2.29±0.15 Ba	2.48±0.15 Ba
SD	0.75±0.06 Aa	0.78±0.05 Aa	1.11±0.09 Bb	1.17±0.07 Bb	1.79±0.10 Aa	1.79±0.13 Aa	1.96±0.16 ABb	2.06±0.08 Bb

Different capital letters in the line for the same elicitors or control indicate statistical differences ($p < 0.05$)
Wartości średnie oznaczone w wierszach dużymi literami różnią się statystycznie istotnie przy $p < 0,05$
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Results obtained in our study showed that treatment of seedlings with elicitors influenced on ability to inhibition of LOX. There were not any differences between activity of hydrophilic and lipophilic extracts of control sprouts. It worth noting that hydrophilic extracts of stimulated seedlings possessed LOXI activity. The best effect was observed after single stimulation by SC 0.1% and SD water extract and three step induction by SC 1% (about 10% of activation). Taking into account lipophilic extracts a slight LOX inhibition (about 5%) was observed after induction by SC 0.1% and one step stimulation by SC 0.5%. In other cases LOXI activity was significantly decreased. The highest decrease was found after single treatment by SC 1% (tab. 2). The inhibition mechanisms of the non-treated and SC 0.1% treated broccoli sprouts extracts were studied by kinetic analysis using double-reciplotal plotting. Lineweaver-Burk plots of reactions in the presence and absence of the testes samples in the LOX reaction mixture are shown in

fig. 1 and 2. Obtained data indicate that extracts from non-treated broccoli sprouts (control sample) inhibited lipoxygenase by acting as noncompetitive inhibitor. The mode of LOX inhibition of hydrophilic inhibitors from treated sprouts is of competitive (fig. 1), whereas lipophilic inhibitors from treated sprouts inhibited LOX by binding either with the free enzyme or the enzyme-substrate complex – mode of inhibition is of mixed type (between uncompetitive and non-competitive type of inhibition) (fig. 2).

They are now known to be metabolites of arachidonic acid formed by the 5-lipoxygenase pathway and are produced almost exclusively by inflammatory leucocytes, especially from mast cells, basophils and eosinophils. The leukotriene synthesis inhibitors block the synthesis of all leukotrienes by interrupting the 5-lipoxygenase pathway. Anti-leukotriene drugs are mainly of two types: cysteinyl leukotriene receptor antagonist and leukotriene synthesis inhibitors (5-Lipoxygenase inhibitor): zileuton, ZD-2138, Bay X 1005, MK-0591. Treatment with oral montelukast, zafirlukast, or zileuton significantly improves many clinical outcomes, including night time awakenings, daytime symptom scores, and uses of beta2 agonists [Samaria 2004]. By the reason of liver disruption possibility Zileuton is not commonly used (inaccessible in Polish) [Kuziemski 2009].

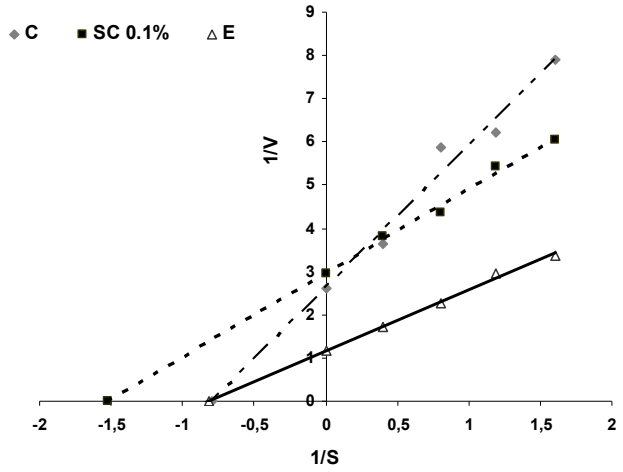

Fig. 1. Mode of LOX inhibition by PBS extracts from broccoli sprouts; C – control sprouts (non-treated), SC 0.1% – sprouts single treated by SC 0.1%, E – sample without extracts

Fig. 1. Mechanizm inhibicji LOX przez buforowe ekstrakty kiełków brokułu; C – kiełki kontrolne (nieindukowane), SC 0.1% – kiełki jednokrotnie indukowane przy użyciu SC 0.1%, E – próba bez ekstraktu

There are some reports concerning potential natural inhibitors of LOX. Yamamoto et al. [2005] showed that (–)-gallocatechin gallate from tea leaves was the potent and selective inhibitor of human platelet 12-lipoxygenase with IC_{50} of 0.14 μM. It was also found that rosemary extract quench soybean 15-lipoxygenase, IC_{50} ranged from 1.3 to

2.6 µg. It should be noted that isoflavone and isoflavone glycosides isolated from kudzu root (i.e. puerarin, daidzin, daidzein, biochanin A and genistein) acted as lipoxygenase inhibitors [Juntachote and Berghofer 2005]. Thyme (*Thymus vulgaris* L. and other species) has a strong traditional use in the treatment of asthma and bronchitis, while basic science has demonstrated that thyme is a potent inhibitor of 5-LOX, which could explain, in part, its beneficial effect on respiratory inflammation [Low Dog 2006]. Satya Prasad et al. [2004] found that lipoxygenase activity was inhibited by compounds present in spices, with the highest level of inhibition was obtained for quercetin (IC_{50} = 25 µM), curcumin (IC_{50} = 28 µM), and eugenol (IC_{50} = 30 µM).

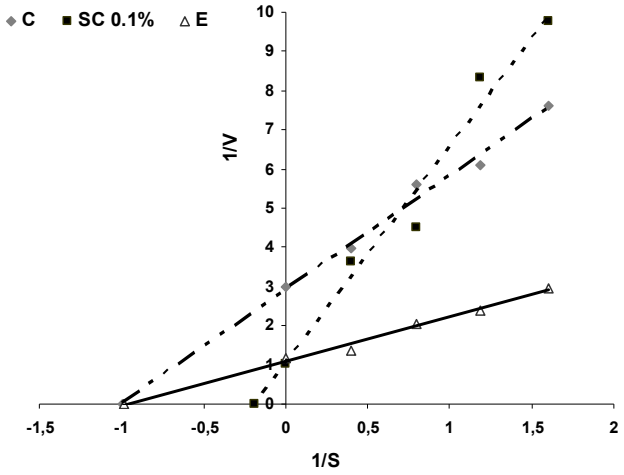

Fig. 2. Mode of LOX inhibition by 50% methanol extracts from broccoli sprouts; C – control sprouts (non-treated), SC 0.1% – sprouts single treated by SC 0.1%, E – sample without extracts

Fig. 2. Mechanizm inhibicji LOX przez metanolowe ekstrakty kiełków brokułu; C – kiełki kontrolne (nieindukowane), SC 0.1% – kiełki jednokrotnie indukowane przy użyciu SC 0.1%, E – próba bez ekstraktu

The xanthine oxidase (XO) is the enzyme that catalyses the metabolism of hypoxanthine and xanthine into uric acid. XO also serves as an important biological source of oxygen-derived free radicals that contribute to oxidative damage to living tissues involved in aging and many pathological processes such as inflammation, atherosclerosis and cancer. Furthermore, significant increments in the serum level of xanthine oxidase are clinically observed in patients who suffer from hepatitis, brain tumor and hyperuricemia and gout. Inhibitors of XO may be potentially useful in the treatment of gout or other XO-induced diseases [Ferraz Filha et al. 2006].

Results obtained in this study indicate that broccoli sprouts were a good source of hydrophilic compounds acted as XO inhibitors (tab. 2). Treatment did not influence significantly on their inhibitory potential (IC_{50} about 1.80 mg/ml).

Very interesting results were obtained in lipophilic extracts case. Activity of control sample was significantly lower than activity of analogical sample obtained after PBS extraction (hydrophilic compounds). In the all experiments a significant increase of XO inhibitory activity was found. The highest elevation was observed after single and three step elicitation by SC1% (2.29 and 2.48 mg/ml, respectively) and SD extract (1.96 and 2.06 mg/ml, respectively) In both cases one step induction was more effective (tab. 2). Hypouricemic agents include XO (critical source of ROS in inflammatory disease) inhibitors are commonly employed for the treatment of XO-involved diseases. In general, allopurinol is the drug of choice, however it has serious side effects [Umamaheswari et al. 2009]. In this reason new alternatives with increased therapeutic activity and lesser side effects are desired.

Lin et al. [2002] confirmed that phenolic compounds are able to inhibit XO. They found that apigenin, genistein and isovitexin (at the concentrations 0.61 μM, 3.23 μM and 5.22 μM respectively) effectively inhibited xanthine oxidase. Similar properties were possessed by quercetin and myrycetin, while naringenin, (with $IC_{50} > 50$ μM) did not decrease XO activity. Ferraz Filha et al. [2006] showed that six *Lychnophora* species traditionally used to treat pain, inflammation and rheumatism exhibited a high XO inhibitory activity (IC_{50} values ≤ 50 μg/ml). Also Sweeney et al. [2001] screened 17 species of Australian native plants used traditionally in the treatment of headache and pains, skin complaints, infected or inflamed eyes, diarrhea and bronchial congestion. The three species of *Clerodendrum floribundum* R. Br., *Eremophila maculata* (Ker Gawler) F. Muell and *Stemodia grossa* Benth exhibited XO inhibitory activity with IC_{50} values less than 50 μg/ml.

There is some evidence that free radicals cause oxidative damage to DNA, proteins, and lipids contributing significantly to aging and degenerative diseases such as cancer, cardiovascular disease, immune system decline, brain dysfunction (e.g. Alzheimer's disease) and cataracts. Antioxidants are compounds that have the ability to scavenge free radicals, especially ROS [Randhir et al. 2004].

In our study, hydrophilic extracts of broccoli sprouts were effective ABTS radicals scavengers. IC_{50} value for control sprouts (without treatment) was 0.11 mg/ml. Induction of sprouts metabolism by natural elicitors caused significant increase of activity in all samples, except of three step treatment by SC1%. The highest increase was found after single treatment with SC 0.1% ($IC_5 = 0.08$ mg/ml). In lipophilic extract cases, the most active samples were obtained after SC0.1% treatment and three step stimulation with SC0.5% (tab. 3).

Another antioxidative mechanism is the chelation of metals such as iron and copper ions, which prevent their participation in Fenton-type reactions and the generation of highly reactive hydroxyl radicals [Cervato et al. 2000]. Broccoli sprouts had hydrophilic compounds with high chelating power. It should be noted that in control samples chelating power of buffer extracts ($IC_5 = 4.14$ mg/ml) was higher then that determined for methanolic extracts ($IC_{50} = 7.16$ mg/ml). In majority of experiments natural elicitor caused a significant decrease of chelating abilities. The highest decrease of activity was observed after single and three step SC0.5% treatment ($IC_5 = 5.55$ mg/ml and 5.39% activity, respectively). In lipophilic compounds (methanolic extracts) only single treatment by SC 1% increased chelating power of broccoli sprouts ($IC_5 = 5.78$ mg/ml),

(tab. 3). Natural extracts were already tested as elicitors of sprouts for improving the antioxidant activity evaluated using β-carotene/linoleate model system. Results obtained by Andarwulan and Shetty [1999] indicate slight effect of acetyl salicylic acid (ASA) on antioxidant activity of pea sprouts. Randhir et al. (2004) stated that treatment of 2-days sprouts of mung bean with 1 ml/l FPH, 500 ppm LF and 10 ml/l OE caused an elevation of the antioxidant activity. Randhir et al. [2002] stated also that antioxidant activity of fava bean sprouts was slightly increased on day 7 and 8 after OE treatment. Cited authors proved that initially phenolic compounds are antioxidant in nature and lower proportions of phenolics are being partitioned for lignification. This may by due to early stages of germination requiring higher demanded for oxygen and therefore phenolics might be protecting the cells from potential oxidation-induced deterioration.

Table 3. Effect of elicitors on antiradical activity and chelating power, expressed as IC_{50} (mg/ml), n = 9

Tabela 3. Wpływ elicitorów na zdolność do neutralizacji wolnych rodników oraz zdolność do chelatowania wyrażona jako IC_{50} (mg/ml), n = 9

Sample próba	Antiradical activity Aktywność przeciwrodnikowa				Chelating power Zdolność do chelatowania			
	hydrophilic hydrofilne		lipophilic lipofilne		hydrophilic hydrofilne		lipophilic lipofilne	
	Ix1	Ix3	Ix1	Ix3	Ix1	Ix3	Ix1	Ix3
Control kontrola	0.11±0.02 Ba	0.11±0.02 Ba	0.48±0.04 Aa	0.48±0.04 Ab	4.14±0.12 Aab	4.14±0.12 Aa	7.16±0.36 Bc	7.16±0.36 Ba
SC 0.1%	0.08±0.01 Aa	0.09±A0.01 Aa	0.36±0.02 Bc	0.38±0.02 Ba	4.33±0.23 Abc	4.56±0.20 Ab	10.13±0.58 Ca	7.60±0.35 Ba
SC 0.5%	0.10±0.01 Aa	0.09±0.01 Aa	0.58±0.05 Cb	0.33±0.01 Bc	5.55±0.31 Ad	5.39±0.22 Ac	18.59±0.97 Cd	11.72±0.88 Bb
SC 1%	0.10±0.01 Aa	0.11±0.00 Aa	0.60±0.05 Cb	0.49±0.02 Bb	3.99±0.26 Aa	4.18±0.09 Aab	5.78±0.69 Bb	7.28±0.56 Ca
SD	0.10±0.01 Aa	0.10±0.00 Aa	0.52±0.02 Ca	0.42±0.02 Ba	4.54±0.19 Ac	4.08±0.18 Aa	8.98±0.49 Ba	11.12±0.86 Cb

Different capital letters in the line for the same elicitors or control indicate statistical differences ($p < 0.05$)
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Different small letters in the columns for the same treatment indicate statistical differences ($p < 0.05$)
Wartości średnie oznaczone w kolumnach małymi literami różnią się statystycznie istotnie przy $p < 0,05$

It is worth noting that generally SC water extract was the most effective elicitor of hydrophilic LOX inhibitors. The different SC concentrations tested were 0.1, 0.5 and 1%. SC extracts proved to be a better elicitor at a low concentration (0.1%). LOXI, XOI and antiradical activity was elevated in both– hydrophilic and lipophilic extracts. Water extract of *Salix daphnoides* bark effectively induced production of lipophilic compounds with XOI activity of and hydrophilic compounds with LOXI. Additionally, it could be seen that in most cases better results were obtained after single induction

(tab. 2). Data presented in table 2 shows that SC 0.1% treatment slightly induced antiradical activity of both (hydrophilic and lipophilic) extracts. It is interesting that elicitation of chelating abilities of broccoli sprouts did not get the expected results. Induction of this activity was observed only for lipophilic extract obtained after single SC 1% treatment (tab. 3).

Natural antioxidants exhibit a wide range of pharmacological activities and have been shown to have anticancer, anti-inflammatory and anti-aging properties. In recent years food biotechnologist are keen to harness the nutritional benefits of these compounds. They have tremendous application in the prevention and treatment of highly prevalent human diseases such as cardiovascular disease, cancer, gastric and duodenal ulcer, allergy, vascular fragility, viral and bacterial infection and Parkinson's disease [Randhir et al. 2002]. In this context the health-beneficial abilities stimulation by natural elicitors was studied. Currently there are no studies about the use of broccoli sprouts as a functional food to treat inflammatory patients. As it can be seen in tables 2 and 3, hydrophilic compounds extracted from broccoli sprouts possessed higher biological activities than lipophilic. It could be suggested that they may be effectively extracted during mastication and thus in gastrointestinal system. It seems reasonable to assume that bioactive compounds from broccoli seeds are bioaccessible and bioavailable in gastrointestinal system. This provides the basic for further research on improving the nutritional and health-beneficent functional value of broccoli sprouts using natural elicitors.

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ZASTOSOWANIE ELICYTORÓW BIOTYCZNYCH W CELU ZWIĘKSZENIA AKTYWNOŚCI ANTYOKSYDACYJNEJ ORAZ ZDOLNOŚCI DO INHIBICJI LIPOKSYGENAZY I OKSYDAZY KSANTYNOWEJ KIEŁKÓW BROKUŁU

Streszczenie. W celu wzmocnienia właściwości prozdrowotnych kiełków brokułu zastosowano elicytację wodnymi ekstraktami drożdży *Saccharomyces cerevisiae* (SC) oraz kory *Salix daphnoides* (SD). Zastosowano roztwory SC w stężeniach 0,1; 0,5 i 1% (w/v) oraz SD w stężeniu 1% (w/v). Indukcja wzrastających kiełków istotnie zmodyfikowała zawartość flawonoidów oraz związków fenolowych ogółem, aktywności antyoksydacyjne oraz zdolność do inhibicji lipoksygenazy (LOXI) i oksydazy ksantynowej (XOI). Zastosowanie 0,1% ekstraktu SC najskuteczniej indukowało aktywność LOXI, XOI oraz aktywność przeciwrodnikową zarówno związków hydrofilnych, jak i lipofilnych. Elicytacja 1% ekstraktem SD efektywnie indukowała aktywność lipofilnych inhibitorów XO oraz hydrofilnych inhibitorów LOX. W większości przypadków najlepsze rezultaty stwierdzono po jednokrotnej indukcji. Zastosowana strategia pozwala na zwiększenie wartości nutraceutycznej żywności nieprzetworzonej, jaką stanowią kiełki brokułu.

Słowa kluczowe: brokuł, elicitory, związki fenolowe, antyoksydanty, inhibicja

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